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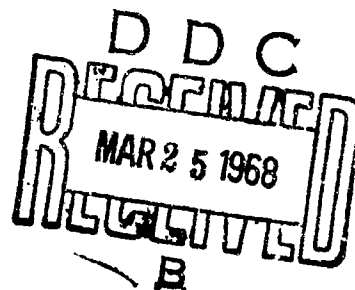
TECHNICAL MANUSCRIPT 428

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MECHANISMS OF HELPER PHAGE - DEPENDENT  
TRANSFECTION IN BACILLUS SUBTILIS

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JANUARY 1968



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MECHANISMS OF HELPER PHAGE - DEPENDENT TRANSFECTION  
IN BACILLUS SUTILIS

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Project 1C014501B71A

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#### ABSTRACT

Bacteriophage SP-18 rendered competent cells of Bacillus subtilis 168 (ind<sup>-</sup>) susceptible to transfection by DNA from apparently unrelated phages. DNA from SP-18 was also active as helper, but it was less effective than the intact phage. Sheared SP-18 DNA retained its helper effect. Phage SP-18 inactivated by ultraviolet irradiation helped SP-10 DNA, but not LP-19 DNA, transfect competent 168 cells.

## I. INTRODUCTION

This report deals with an investigation into the mechanism of the helper effect observed with bacteriophage SP-18 that renders competent cells of Bacillus subtilis 168 (ind<sup>-</sup>) susceptible to transfection by DNA from apparently unrelated phages.<sup>1</sup> When SP-18 DNA was sheared to the extent that SP-18 infectious centers were no longer recovered from a mixture composed of sheared SP-18 DNA, nonsheared LP-19 DNA, nonsheared SP-10 DNA, and competent 168 cells, infectious centers of LP-19 and SP-10 were still recovered. Phage SP-18 inactivated 99% by ultraviolet irradiation was active as helper when mixed with SP-10 DNA and added to competent 168 cells, but it was not active as helper when the same procedure was followed using LP-19 DNA.

Phage SP-10 adsorbs to and injects its DNA into 168 cells but it will not propagate or form infectious centers in these cells.<sup>2</sup> The helper effect of phage SP-18 was utilized in an attempt to determine the fate of SP-10 DNA inside a 168 cell. Cells of 168 infected with SP-10 produced SP-10 infectious centers when they were subsequently infected with SP-18 phage. By use of this technique, we showed that SP-10 genomes remain biologically active within 168 cells for a longer time under static conditions than under aerated conditions.

## II. MATERIALS AND METHODS

Phages SP-18 and LP-19 were isolated from soil by Dr. Ivan D. Goldberg in this laboratory. SP-10 is the transducing phage described by Thorne.<sup>3</sup> All phage suspensions were assayed on phage assay (PA) agar seeded with spores of the appropriate indicator as described by Thorne.<sup>3</sup> SP-10 was propagated on E. subtilis W-23-S<sup>r</sup> according to Taylor and Thorne.<sup>4</sup> Shaken cultures in TY broth<sup>5</sup> were used for preparing SP-18 lysates on E. subtilis W-23-S<sup>r</sup> and LP-19 lysates on Bacillus licheniformis 9945A.

Phage DNA was extracted by a modification of the phenol technique of Mandell and Hershey.<sup>6</sup> The DNA was dialyzed for 22 hours at 5 C against 0.15 M NaCl - 0.015 M citrate (SSC) at pH 8.0 to remove the phenol and stored at 5 C. DNA was determined by the method of Burton.<sup>6</sup> All DNA preparations were tested for contamination with bacteria or viable phage by spreading samples on nutrient agar and by plating samples (after treatment with 50 µg of deoxyribonuclease) in PA agar seeded with the appropriate indicator.

Competent cells of *B. subtilis* 168 (ind<sup>-</sup>) were grown by essentially the same procedure as that of Anagnostopoulos and Spizizen.<sup>7</sup> Transfections were carried out as described by Gwinn and Thorne.<sup>1</sup>

### III. RESULTS AND DISCUSSION

SP-18 phage inactivated 99% by ultraviolet (UV) irradiation retained the ability to help SP-10 DNA transfect competent *B. subtilis* 168 cells, but UV-inactivated SP-18 would not help LP-19 DNA transfect competent 168 cells (Table 1). The ratio of LP-19 infectious centers obtained using nonirradiated SP-18 versus irradiated SP-18 as helper was 37:1, but the ratio of infectious centers obtained for SP-10 was 1.6:1. The fact that transfection of competent 168 cells with LP-19 DNA was not helped by UV-inactivated SP-18 phage, although transfection of competent 168 cells with SP-10 DNA was helped, suggests that more than one marker on the SP-18 genome may be required in helping LP-19 DNA transfect competent 168 cells.

TABLE 1. HELPER EFFECT OF SP-18 PHAGE INACTIVATED BY ULTRAVIOLET IRRADIATION<sup>a/</sup>

MOI <sup>b/</sup> of Viable SP-18		DNA, $\mu$ g/ml		Infectious Centers/ml	
- UV	+ UV	LP-19	SP-10	LP-19	SP-10
0	0	0	0	0	0
0.6	0	91	0	$6.0 \times 10^4$	0
0	0.006	91	0	$1.6 \times 10^3$	0
0.005	0	112	0	$1.0 \times 10^3$	0
0.6	0	0	88	0	$1.6 \times 10^3$
0	0.006	0	88	0	$1.0 \times 10^3$
0.012	0	0	97	0	$1.0 \times 10^1$

a. Four ml of SP-18 lysate contained in a glass petri dish were irradiated for 45 sec on an oscillating platform with two 15-watt (GE) germicidal lamps at a distance of 50 cm.

Recipient cells ( $2 \times 10^8$ ), DNA, and SP-18 phage as given were incubated together in a final volume of 1 ml for 60 min. Following treatment with deoxyribonuclease, the mixtures were assayed for infectious centers in M10 agar seeded with spores of *B. licheniformis* 9945A. To assist in scoring LP-19 and SP-10 infectious centers, SP-18 antiserum was added to the plating mixtures to inactivate residual free SP-18 as well as that released from SP-18 infectious centers.

b. MOI = multiplicity of infection.



When SP-18 DNA was sheared to the extent that SP-18 infectious centers could no longer be recovered from a mixture composed of sheared SP-18 DNA, SP-10 DNA or LP-19 DNA, and competent 168 cells, infectious centers of SP-10 and LP-19 were still produced (Fig. 1). The absence of SP-18 infectious centers in the mixtures containing 2X sheared SP-18 DNA and SP-10 DNA or LP-19 DNA was probably due to competition between the DNA of the two phages to be taken up by competent 168 cells,<sup>1,8</sup> resulting in not enough pieces of SP-18 genome getting into a 168 cell to recombine and form a complete SP-18 genome. It is unlikely that SP-10 and LP-19 infectious centers obtained from mixtures containing 2X sheared SP-18 DNA (Fig. 1) resulted from the helper effect of complete SP-18 genomes that had escaped being broken. Davison<sup>9</sup> showed that when phage T<sub>4</sub> DNA (T<sub>4</sub> and SP-18 are approximately the same size) was sheared with a force only one-fifth as great as that used for 2X sheared SP-18 DNA, the sedimentation coefficient of the T<sub>4</sub> DNA was lowered from approximately 60S to approximately 35S. According to Davison, "theoretical calculations show that the rupturing stress applied by the viscous forces is maximal in the center of the chain and increases as the square of the length of the molecules. The molecules should thus break roughly in half when the critical shear is applied, and a very long molecule should be halved repeatedly until a fairly uniform population of chains stable to the shear gradient is obtained." When long DNA molecules were subjected to a shearing force, the formation of "half" and "quarter" molecules of DNA was substantiated by Burgi and Hershey<sup>10</sup> and by Green.<sup>11</sup> The dose-response curves for sheared and nonsheared SP-18 DNA are given in Figure 2. The slope of the curves resulting from sheared SP-18 DNA was approximately 2.5; the slope of the curve resulting from nonsheared SP-18 DNA was approximately 1.0. These results indicate that (i) the interaction of a single SP-18 DNA molecule with a competent 168 cell resulted in an infectious center when nonsheared SP-18 DNA was used and (ii) the cooperation of more than one DNA molecule was required for the establishment of an infectious center when 2X or 3X sheared SP-18 DNA was used.<sup>8,12</sup> As the concentration of the nonsheared SP-18 DNA was lowered, the slope of the dose-response curve increased to approximately 2.5. The increase in slope was probably caused by the sensitivity of high molecular weight DNA to shear degradation in dilute solution.<sup>9</sup> SP-18 and LP-19 infectious centers were recovered from a mixture containing diluted SP-18 DNA (0.8 µg/ml), LP-19 DNA (83 µg/ml), and competent 168 cells, but no SP-18 infectious centers were obtained (Fig. 1) from a mixture of 2X sheared SP-18 DNA (100 µg/ml), LP-19 DNA (83 µg/ml), and competent cells. These results indicate that helper-dependent transfection can be initiated by the presence of only a small piece of SP-18 genome within a competent 168 cell.

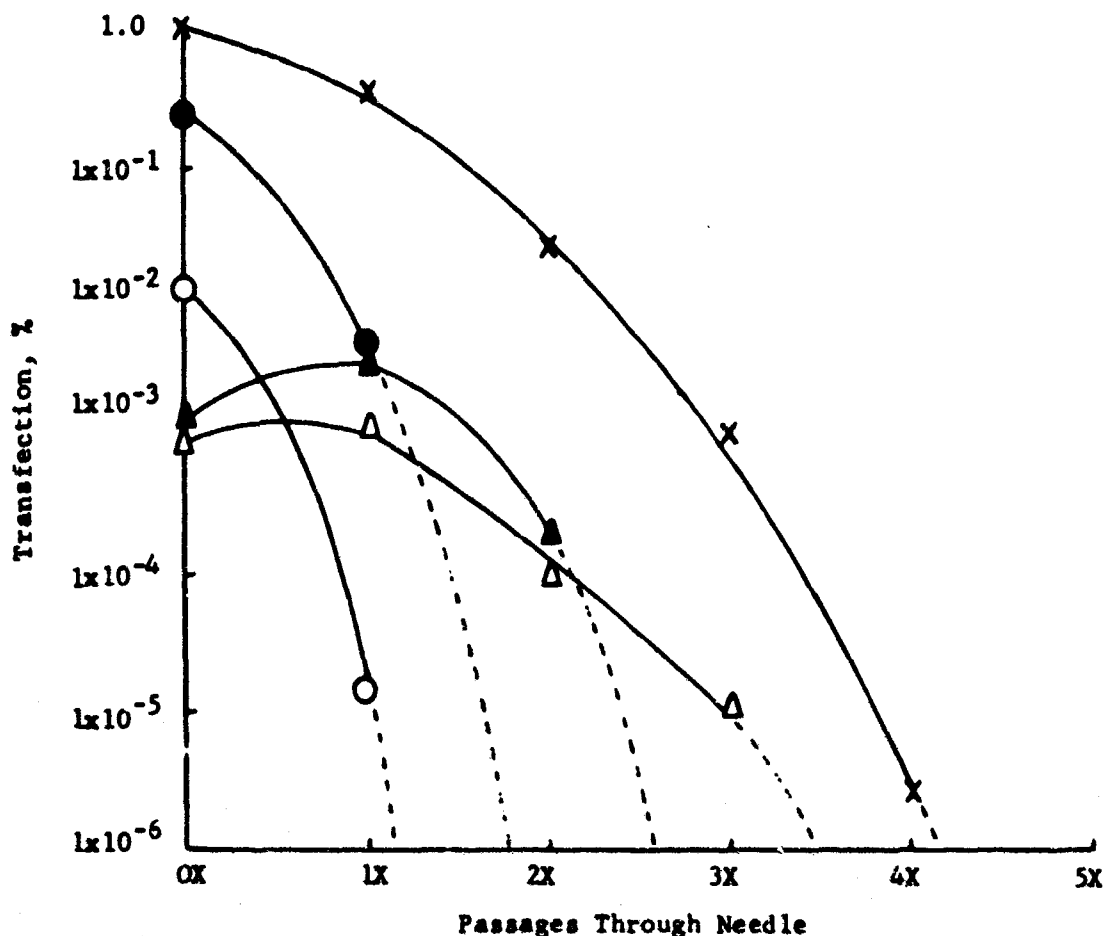


Figure 1. Helper Effect of Sheared SP-18 DNA. SP-18 DNA at a concentration of 2,000  $\mu\text{g}/\text{ml}$  was sheared by being forced through a 26-gauge needle at a rate of 1 ml/2 sec. Recipient cells ( $2 \times 10^8$ ), LP-19 DNA (83  $\mu\text{g}$ ) or SP-10 DNA (70  $\mu\text{g}$ ), and SP-18 DNA (100  $\mu\text{g}$ ) were incubated together in a final volume of 1 ml for 60 min. Following treatment with deoxyribonucleases, the mixtures were assayed for infectious centers in M10 agar seeded with spores of *B. subtilis* 168\* (for SP-18) or *B. licheniformis* 9945A (for SP-10 and LP-19). Controls in which phage DNA was treated with deoxyribonucleases before it was added to recipient cells produced no infectious centers.

- X Per cent SP-18 transfection from SP-18 DNA only.
- Per cent SP-18 transfection from a mixture of SP-18 DNA and LP-19 DNA
- Per cent SP-18 transfection from a mixture of SP-18 DNA and SP-10 DNA.
- △ Per cent LP-19 transfection from a mixture of SP-18 DNA and LP-19 DNA.
- ▲ Per cent SP-10 transfection from a mixture of SP-18 DNA and SP-10 DNA.

\* This culture was derived from a colony that had reverted spontaneously to prototrophy.

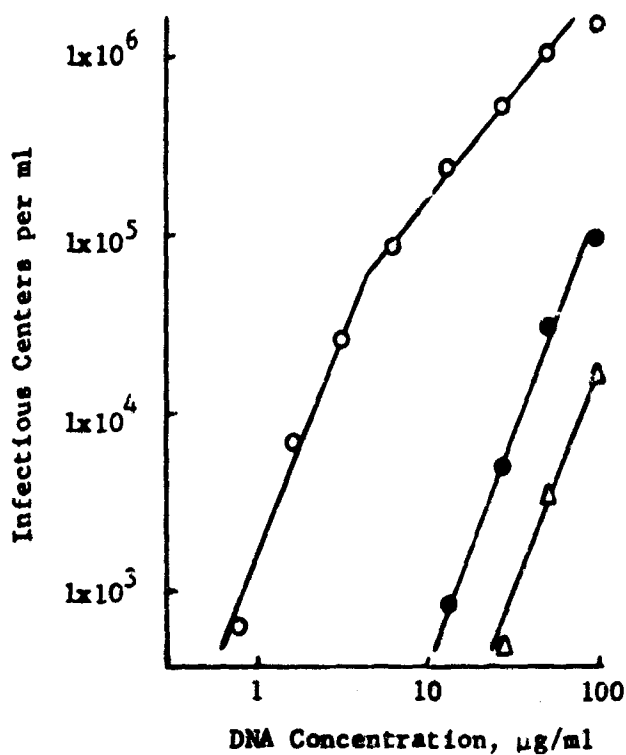


Figure 2. Effect of Sheared SP-18 DNA Concentration on Infectious Center Formation. Each curve results from a serial twofold dilution of DNA in SSC, pH 8.0. Competent 168 cells ( $2 \times 10^8$ /ml) were incubated with each DNA sample for 45 min at 37 C, and infectious centers were assayed.  $\circ$  = non-sheared SP-18 DNA,  $\bullet$  = 2X sheared SP-18 DNA,  $\Delta$  = 3X sheared SP-18 DNA.

To determine if the SP-10 DNA was degraded within 168 host cells, we performed experiments in which 168 cells were infected with SP-10 phage and permitted to incubate for 90 min before SP-18 helper phage was added in order to rescue any intact SP-10 genomes and permit them to develop into SP-10 infectious centers (Fig. 3). One per cent of the original number of SP-10 infectious centers were rescued from 168 cells incubated with aeration, but 7% were rescued when a sample of the same infection mixture was incubated statically. The rescue of SP-10 infectious centers from SP-10-infected 168 cells indicated that a significant number of biologically active SP-10 genomes survived within 168 cells. The latent period for SP-10 in W-23-S<sup>r</sup> is 55 minutes,<sup>13</sup> and, assuming that SP-10 has a similar latent period in 168, the degradation of SP-10 DNA within 168 cells was probably not responsible for the inability of SP-10 to form infectious centers. To determine the need for helper SP-18 phage for uptake of SP-10 DNA or LP-19 DNA, each DNA was incubated with competent 168 cells, and, at various intervals, samples were treated with deoxyribonuclease and infected with SP-18 phage. Because we were able to rescue biologically active SP-10 or LP-19 genomes within the 168 cells, we concluded that the helping effect of SP-18 was not necessary for the uptake of SP-10 DNA or LP-19 DNA into competent 168 cells.

The mechanisms of helper effect that we are considering are: (i) SP-18 could produce some product that permits SP-10 or LP-19 to develop within 168 cells, comparable to the finding of Kozinski and Kozinski<sup>14</sup> that a DNA polymerase produced by T<sub>4</sub> phage was able to replicate the DNA of a polymerase-negative mutant; (ii) SP-18 could neutralize a repressor produced by 168 that prevents the development of SP-10 or LP-19; and (iii) SP-10 or LP-19 may find it impossible to compete in actively metabolizing 168 cells. SP-18 could inhibit the host metabolism and thus permit maturation of SP-10 or LP-19. We believe the latter alternative to be unlikely because SP-3, which we found to be as efficient a helper as SP-18, does not inhibit the metabolism of 168 cells.\*

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\* Romig, W.R., personal communication.

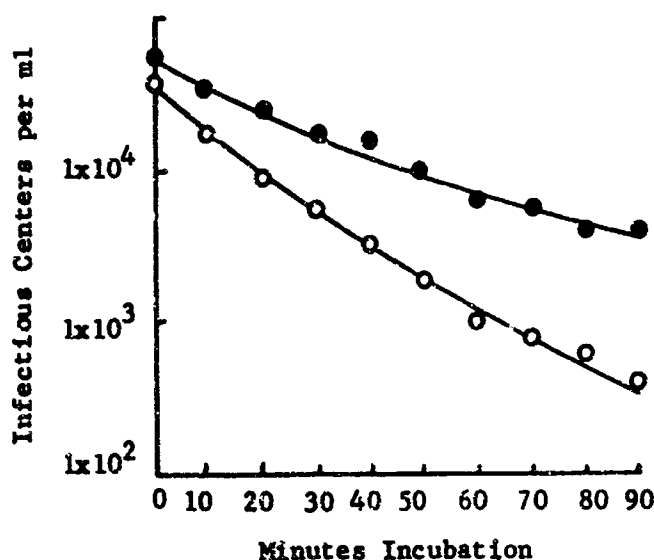


Figure 3. Rescue of SP-10 Phage from *B. subtilis* 168 Cells with SP-18 Helper Phage. Cells of *B. subtilis* 168 were infected with SP-10 phage at an MOI of 2. Residual SP-10 phage was inactivated with SP-10 antiserum. One-half of the infected cells was incubated with aeration (○-○), and the other half was incubated statically (●-●). At 10-min intervals, 1-ml samples were infected with SP-18 phage at an initial MOI of 16 and incubated for 60 min. The samples were assayed for infectious centers in M10 agar seeded with spores of *B. licheniformis* 9945A. To assist in scoring SP-10 infectious centers, SP-18 antiserum was added to the plating mixtures to inactivate residual free SP-18 as well as that released from SP-18 infectious centers.

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